Fig. 1

A

Signal sequence

MVHATSPLLL LLLLSLALVA PGLSARKRTQ

β5 β6
PTFGFTVNWK FSESTTVFTG QCFIDRNGKE

β7 β8
VLKTMWLLRS SVNDIGDDWK ATRVGINIFT

β1
RLRTQKEGGS GGSARKCSLT GKWTNDLGSN

β2 β3
MTIGAVNSRG EFTGTYITAV TATSNEIKES

β4 β6
PLHGTQNTIN KSGGSTTVFT GQCFIDRNGK

β7 β8
EVLKTMWLLR SSVNDIGDDW KATRVGINIF

β1
TRLRTQKEGG SGGSARKCSL TGKWTNDLGS

β2 β3
NMTIGAVNSR GEFTGTYITA VTATSNEIKE

β4 β5
SPLHGTQNTI NKRTQPTFGF TVNWKFSE

B

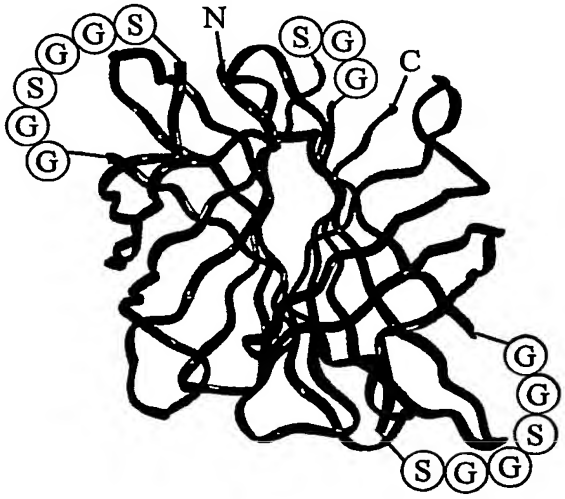

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Fig. 2

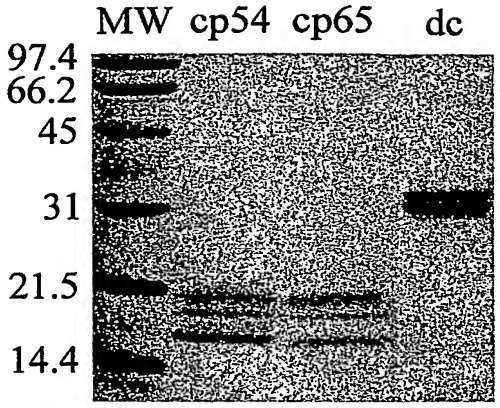


Fig. 3

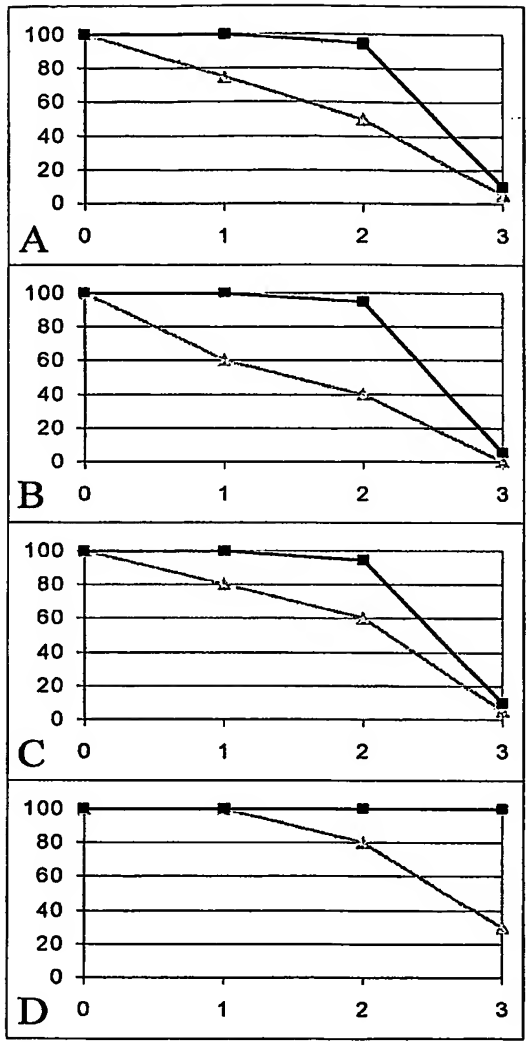


Fig. 4

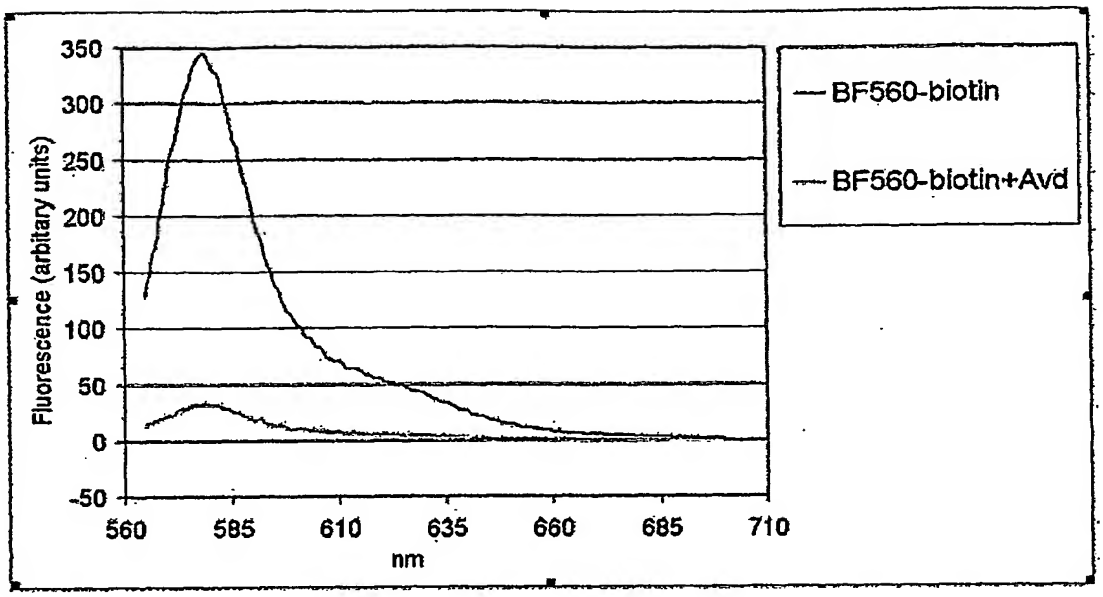

350
300
250
200
150
100
50
0
-50
Fluorescence (arbitary units)
560
585
610
635
660
685
710
nm
BF560-biotin
BF560-biotin+Avd

Fig. 5

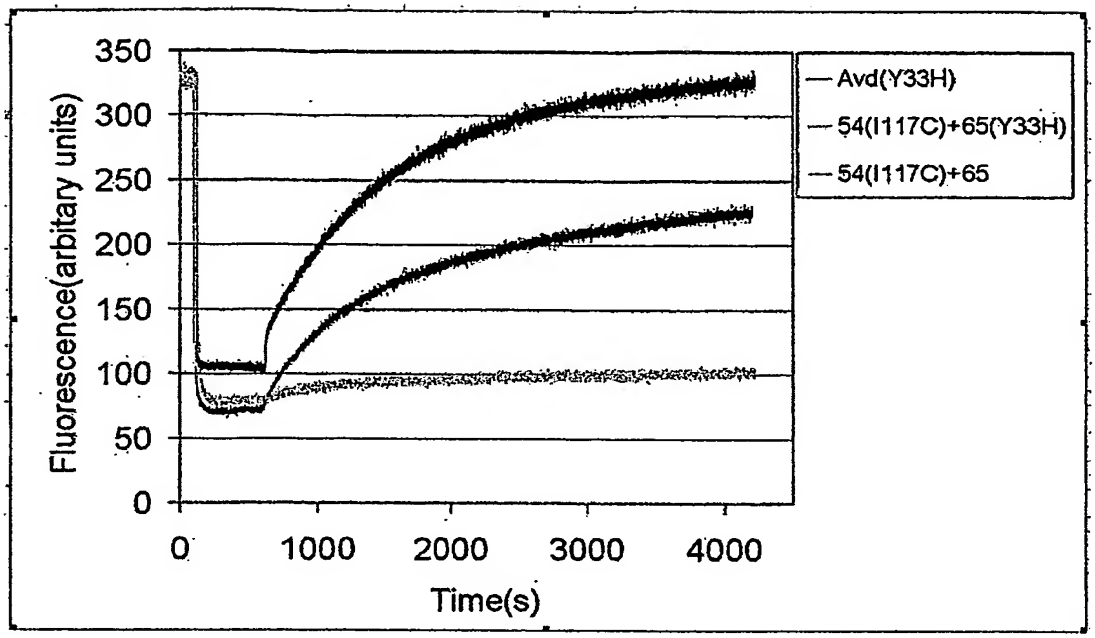

350
300
250
200
150
100
50
0
Fluorescence(arbitary units)
0
1000
2000
3000
4000
Time(s)
Avd(Y33H)
54(I117C)+65(Y33H)
54(I117C)+65

Fig. 6

ATGGTGCACGCAACCTCCCCGCTGCTGCTGCTGCTGCT
GCTCAGCCTGGCTCTGGTGGCTCCCGGCCTCTCTGCCA
GGAAGAGGACCCAGCCCACCTTTGGCTTCACCGTCAAT
TGGAAGTTTTCAGAGTCCACCACTGTCTTCACGGGCCA
GTGCTTCATAGACAGGAATGGGAAGGAGGTCCTGAAG
ACCATGTGGCTGCTGCGGTCAAGTGTTAATGACATTGG
TGATGACTGGAAAGCTACCAGGGTCGGCATCAACATC
TTCACTCGCCTGCGCACACAGAAGGAGGGAGGCTCCG
GAGGCTCCGCCAGAAAGTGCTCGCTGACTGGGAAATG
GACCAACGATCTGGGCTCCAACATGACCATCGGGGCT
GTGAACAGCAGAGGTGAATTCACAGGCACCTACATCA
CAGCCGTAACAGCCACATCAAATGAGATCAAAGAGTC
ACCACTGCATGGGACACAAAACACCATCAACAAGTCC
GGCGGATCCACCACTGTCTTCACGGGCCAGTGCTTCAT
AGACAGGAATGGGAAGGAGGTCCTGAAGACCATGTGG
CTGCTGCGGTCAAGTGTTAATGACATTGGTGATGACTG
GAAAGCTACCAGGGTCGGCATCAACATCTTCACTCGCC
TGCGCACACAGAAGGAGGGAGGCTCCGGAGGCTCCGC
CAGAAAGTGCTCGCTGACTGGGAAATGGACCAACGAT
CTGGGCTCCAACATGACCATCGGGGCTGTGAACAGCA
GAGGTGAATTCACAGGCACCTACATCACAGCCGTAAC
AGCCACATCAAATGAGATCAAAGAGTCACCACTGCAT
GGGACACAAAACACCATCAACAAGAGGACCCAGCCCA
CCTTTGGCTTCACCGTCAATTGGAAGTTTTCAGAGGGA
GGTTCCGGATCGGGATCCGGCTCTGGCAGCGGCAGGA
CCCAGCCCACCTTTGGCTTCACCGTCAATTGGAAGTTT
TCAGAGTCCACCACTGTCTTCACGGGCCAGTGCTTCAT
AGACAGGAATGGGAAGGAGGTCCTGAAGACCATGTGG
CTGCTGCGGTCAAGTGTTAATGACATTGGTGATGACTG
GAAAGCTACCAGGGTCGGCATCAACATCTTCACTCGCC
TGCGCACACAGAAGGAGGGAGGCTCCGGAGGCTCCGC
CAGAAAGTGCTCGCTGACTGGGAAATGGACCAACGAT
CTGGGCTCCAACATGACCATCGGGGCTGTGAACAGCA
GAGGTGAATTCACAGGCACCTACATCACAGCCGTAAC
AGCCACATCAAATGAGATCAAAGAGTCACCACTGCAT
GGGACACAAAACACCATCAACAAGTCCGGCGGATCCA
CCACTGTCTTCACGGGCCAGTGCTTCATAGACAGGAAT
GGGAAGGAGGTCCTGAAGACCATGTGGCTGCTGCGGT
CAAGTGTTAATGACATTGGTGATGACTGGAAAGCTAC
CAGGGTCGGCATCAACATCTTCACTCGCCTGCGCACAC
AGAAGGAGGGAGGCTCCGGAGGCTCCGCCAGAAAGTG
CTCGCTGACTGGGAAATGGACCAACGATCTGGGCTCC
AACATGACCATCGGGGCTGTGAACAGCAGAGGTGAAT
TCACAGGCACCTACATCACAGCCGTAACAGCCACATC
AAATGAGATCAAAGAGTCACCACTGCATGGGACACAA
AACACCATCAACAAGAGGACCCAGCCCACCTTTGGCT
TCACCGTCAATTGGAAGTTTTCAGAGTGA

Fig. 7

ATGGTGCACGCAACCTCCCCGCTGCTGCTGCTGCTGCTGCTCA
GCCTGGCTCTGGTGGCTCCCGGCCTCTCTGCCAGGAAGAGGAC
CCAGCCCACCTTTGGCTTCACCGTCAATTGGAAGTTTTCAGAG
TCCACCACTGTCTTCACGGGCCAGTGCTTCATAGACAGGAATG
GGAAGGAGGTCCTGAAGACCATGTGGCTGCTGCGGTCAAGTGT
TAATGACATTGGTGATGACTGGAAAGCTACCAGGGTCGGCATC
AACATCTTCACTCGCCTGCGCACACAGAAGGAGGGAGGCTCCG
GAGGCTCCGCCAGAAAGTGCTCGCTGACTGGGAAATGGACCAA
CGATCTGGGCTCCAACATGACCATCGGGGCTGTGAACAGCAGA
GGTGAATTCACAGGCACCTACATCACAGCCGTAACAGCCACAT
CAAATGAGATCAAAGAGTCACCACTGCATGGGACACAAAACAC
CATCAACAAGTCCGGCGGATCCACCACTGTCTTCACGGGCCAG
TGCTTCATAGACAGGAATGGGAAGGAGGTCCTGAAGACCATGT
GGCTGCTGCGGTCAAGTGTTAATGACATTGGTGATGACTGGAA
AGCTACCAGGGTCGGCATCAACATCTTCACTCGCCTGCGCACA
CAGAAGGAGGGAGGCTCCGGAGGCTCCGCCAGAAAGTGCTCGC
TGACTGGGAAATGGACCAACGATCTGGGCTCCAACATGACCAT
CGGGGCTGTGAACAGCAGAGGTGAATTCACAGGCACCTACATC
ACAGCCGTAACAGCCACATCAAATGAGATCAAAGAGTCACCAC
TGCATGGGACACAAAACACCATCAACAAGAGGACCCAGCCCAC
CTTTGGCTTCACCGTCAATTGGAAGTTTTCAGAGTGA

Fig. 8

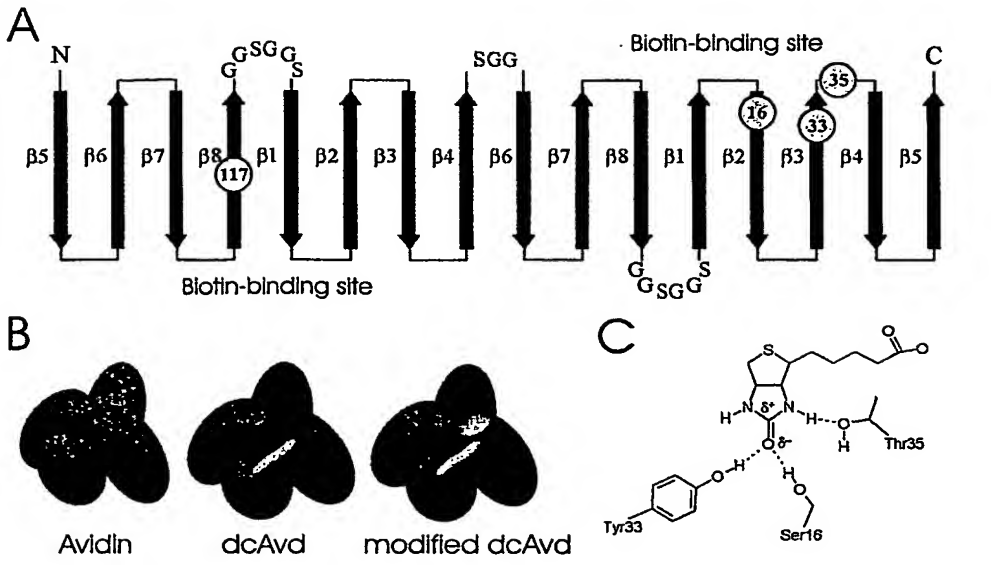

A
N
GSGGS
SGG
Biotin-binding site
C
β5 β6 β7 β8 β1 β2 β3 β4 β6 β7 β8 β1 β2 β3 β4 β5
117
16
33
35
Biotin-binding site
GSGGS
B
C
Avidin
dcAvd
modified dcAvd
Thr35
Tyr33
Ser16

Fig. 9

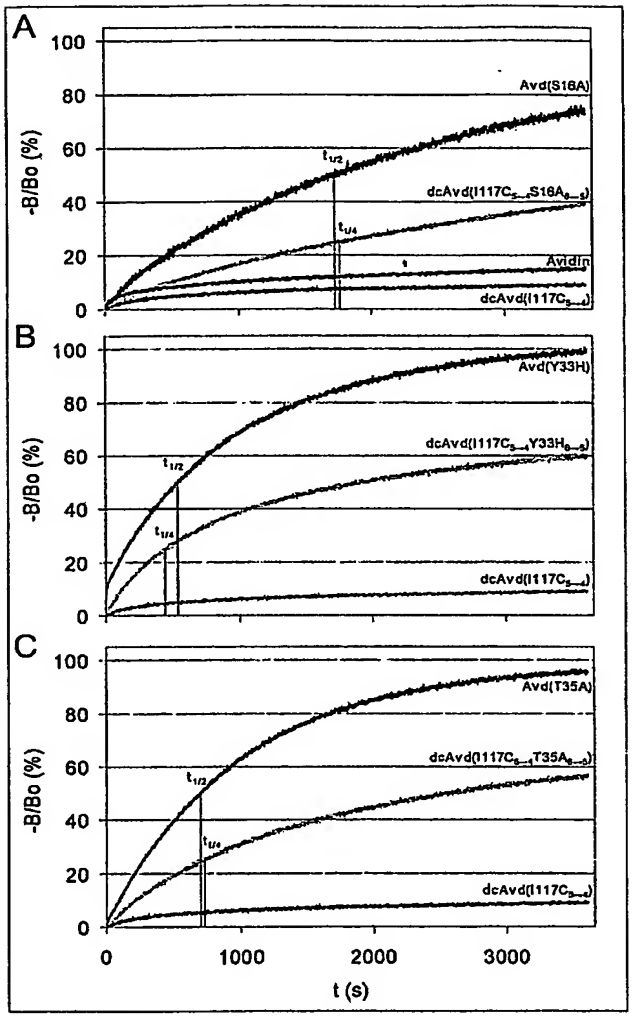

A
B
C
-B/Bo (%)
100
80
60
40
20
0
Avd(S16A)
dcAvd(I117C$_{s-1}$S16A$_{s-4}$)
Avd(n
dcAvd(I117C$_{s-4}$)
Avd(Y33H)
dcAvd(I117C$_{s-1}$Y33H$_{s-4}$)
dcAvd(I117C$_{s-4}$)
Avd(T35A)
dcAvd(I117C$_{s-1}$T35A$_{s-4}$)
dcAvd(I117C$_{s-4}$)
$t_{1/2}$
$t_{1/4}$
0
1000
2000
3000
t (s)

FIG. 10

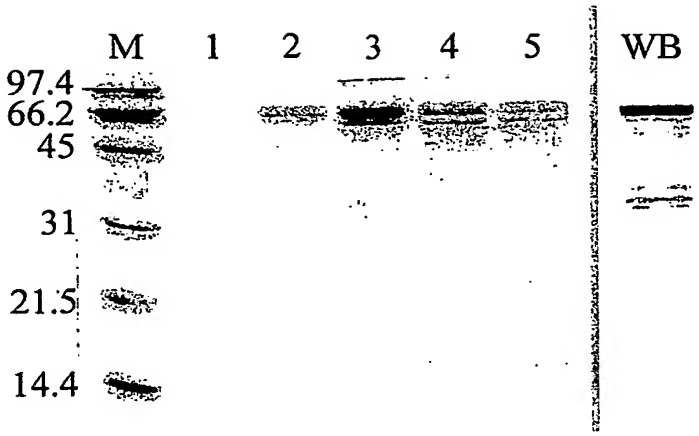


FIG. 11

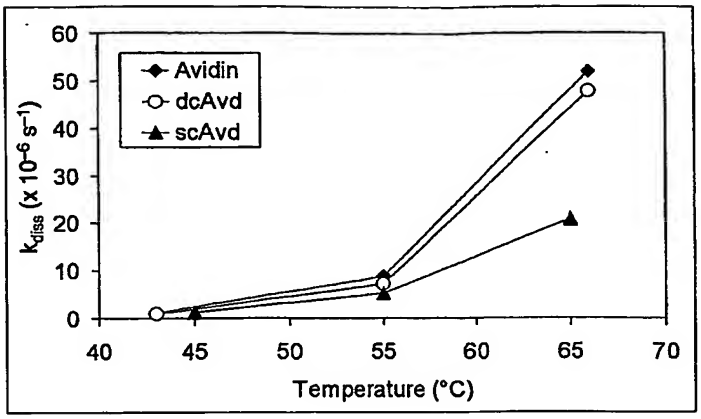

Avidin
dcAvd
scAvd
k_{diss} (x 10^{-6} s^{-1})
0
10
20
30
40
50
60
40
45
50
55
60
65
70
Temperature (°C)

FIG. 12

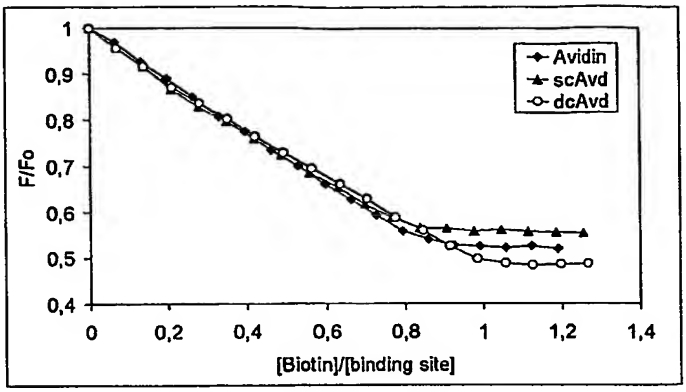

Avidin
scAvd
dcAvd
F/Fo
1
0,9
0,8
0,7
0,6
0,5
0,4
0
0,2
0,4
0,6
0,8
1
1,2
1,4
[Biotin]/[binding site]

FIG. 13

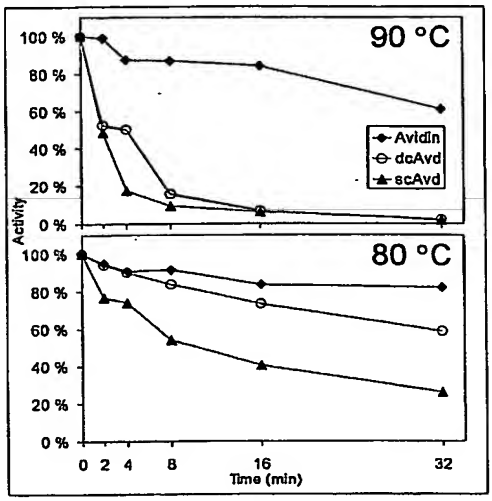
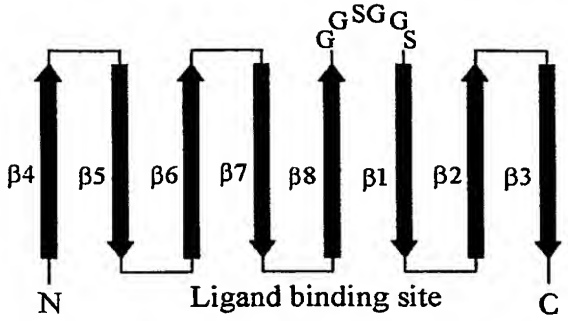

90 °C
80 °C
Avidin
dcAvd
scAvd
Activity
100 %
80 %
60 %
40 %
20 %
0 %
0 2 4 8 16 32
Time (min)

Fig. 14



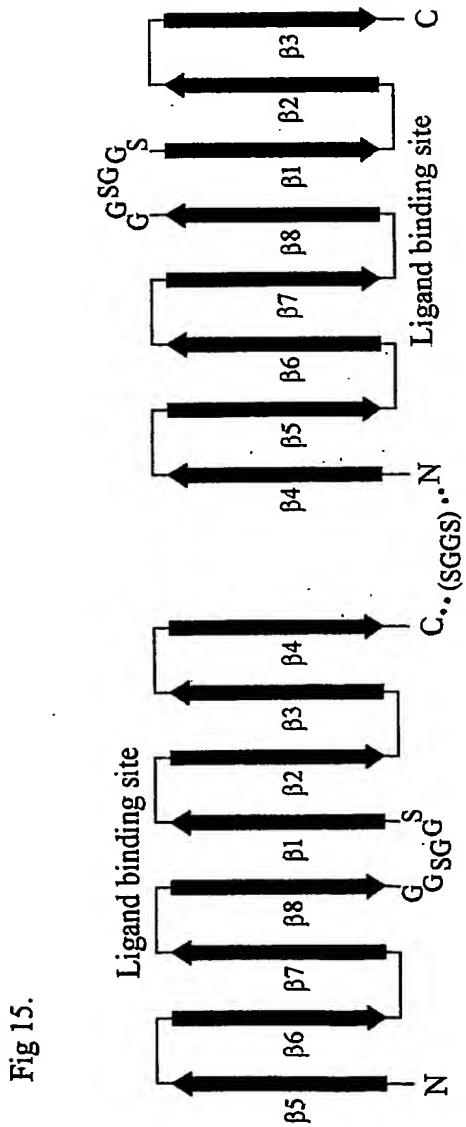

β5
β6
β7
β8
Ligand binding site
G
G
S
β1
β2
β3
β4
C
(SGGS)
N
β4
β5
β6
β7
β8
Ligand binding site
G
G
S
β1
β2
β3
C
G
G
S
N

Fig 15.

Fig. 16

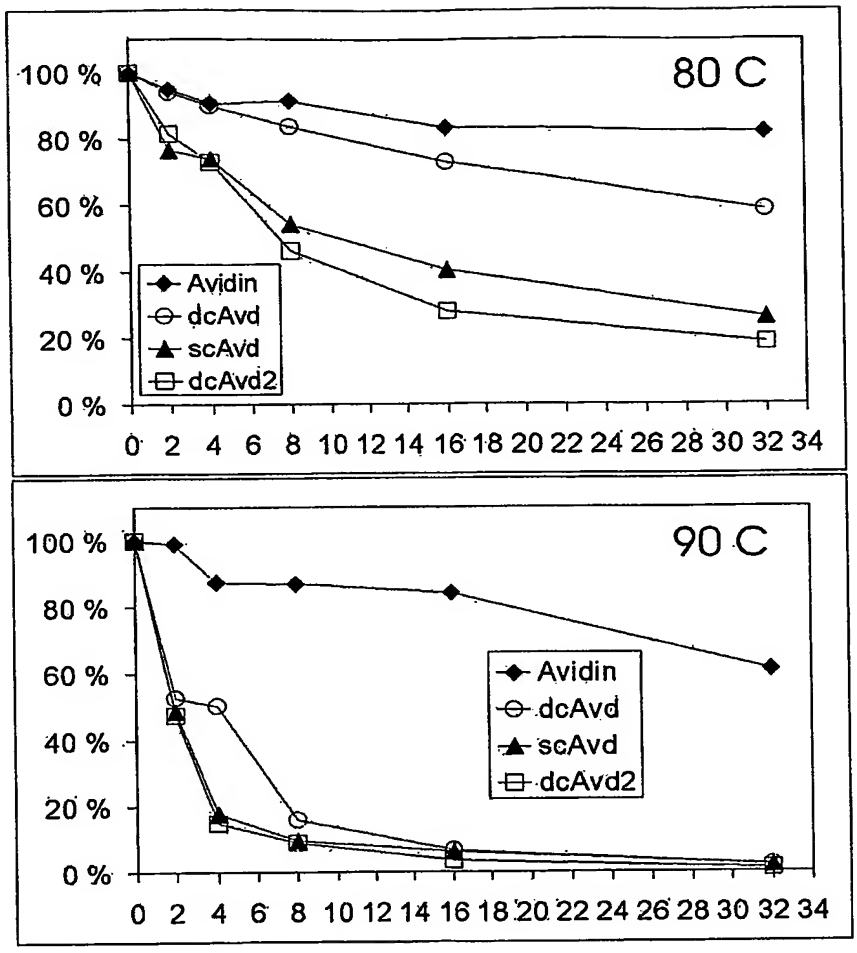

80 C
100 %
80 %
60 %
40 %
20 %
0 %
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
Avidin
dcAvd
scAvd
dcAvd2
90 C
100 %
80 %
60 %
40 %
20 %
0 %
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
Avidin
dcAvd
scAvd
dcAvd2

Fig. 17

```
1       MNKPSKFALP LAFAAVTASG VASAGTQPTF GFTVNWKFSE
STTVFTGQCF IDRNGKEVLK
61      TMWLLRSSVN DIGDDWKATR VGINIFTRLR TQKEGGSGGS
ARKCSLTGKW TNDLGSNMTI
121     GAVNSRGEFT GTYITAVTAT SNEIKESPLH GTQNTINKSG
GSKESPLHGT QNTINKRTQP
181     TFGFTVNWKF SESTTVFTGQ CFIDRNGKEV LKTMWLLRSS
VNDIGDDWKA TRVGINIFTR
241     LRTQKEGGSG GSARKCSLTG KWTNDLGSNM TIGAVNSRGE
        FTGTYITAVT
```

Fig. 18

```
1       ATGAACAAAC CCTCCAAATT CGCTCTGCCG CTTGCCTTCG
CCGCCGTTAC GGCCTCTGGT
61      GTTGCCTCGG CTGGTACCCA GCCCACCTTT GGCTTCACCG
TCAATTGGAA GTTTTCAGAG
121     TCCACCACTG TCTTCACGGG CCAGTGCTTC ATAGACAGGA
ATGGGAAGGA GGTCCTGAAG
181     ACCATGTGGC TGCTGCGGTC AAGTGTTAAT GACATTGGTG
ATGACTGGAA AGCTACCAGG
241     GTCGGCATCA ACATCTTCAC TCGCCTGCGC ACACAGAAGG
AGGGAGGCTC CGGAGGCTCC
301     GCCAGAAAGT GCTCGCTGAC TGGGAAATGG ACCAACGATC
TGGGCTCCAA CATGACCATC
361     GGGGCTGTGA ACAGCAGAGG TGAATTCACA GGCACCTACA
TCACAGCCGT AACAGCCACA
421     TCAAATGAGA TCAAAGAGTC ACCACTGCAT GGGACACAAA
ACACCATCAA CAAGTCCGGC
481     GGATCCAAAG AGTCACCACT GCATGGGACA CAAAACACCA
TCAACAAGAG GACCCAGCCC
541     ACCTTTGGCT TCACCGTCAA TTGGAAGTTT TCAGAGTCCA
CCACTGTCTT CACGGGCCAG
601     TGCTTCATAG ACAGGAATGG GAAGGAGGTC CTGAAGACCA
TGTGGCTGCT GCGGTCAAGT
661     GTTAATGACA TTGGTGATGA CTGGAAAGCT ACCAGGGTCG
GCATCAACAT CTTCACTCGC
721     CTGCGCACAC AGAAGGAGGG AGGCTCCGGA GGCTCCGCCA
GAAAGTGCTC GCTGACTGGG
781     AAATGGACCA ACGATCTGGG CTCCAACATG ACCATCGGGG
CTGTGAACAG CAGAGGTGAA
841     TTCACAGGCA CCTACATCAC AGCCGTAACA TAA
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